

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
18 December 2003 (18.12.2003)

PCT

(10) International Publication Number  
**WO 03/104470 A2**

(51) International Patent Classification<sup>7</sup>: C12N 15/90,  
9/12, 15/11, 15/79, A61K 48/00

(21) International Application Number: PCT/CA03/00850

(22) International Filing Date: 5 June 2003 (05.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/386,640 5 June 2002 (05.06.2002) US

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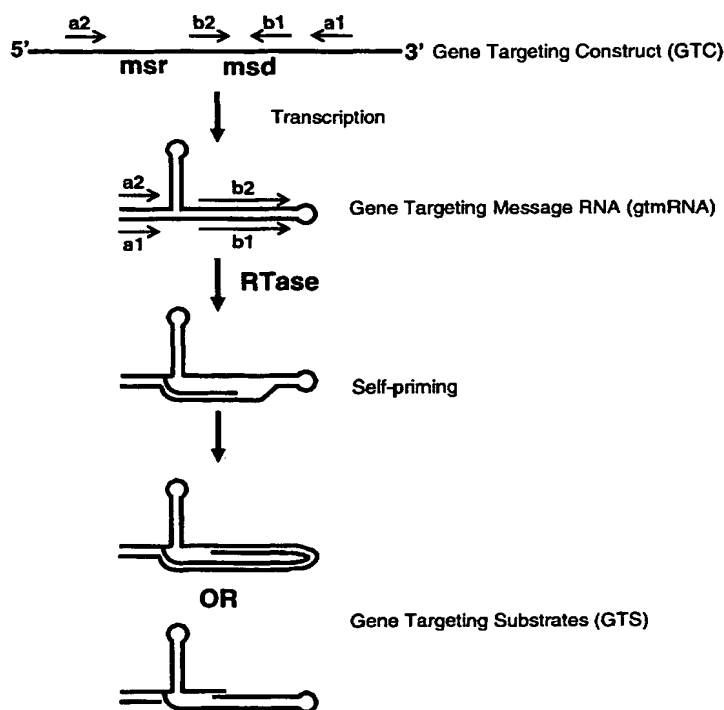
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(81) Designated States (*national*): AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,

[Continued on next page]

(54) Title: RETRONS FOR GENE TARGETING



(57) Abstract: The invention provides methods and nucleic acid constructs that may be used to modify a nucleic acid of interest at a target locus within the genome of a host. In some aspects, the invention contemplates producing *in vivo* a gene targeting substrate (GTS), which may be comprised of both DNA and RNA components. The gene targeting substrate may comprise a gene targeting nucleotide sequence (GTNS), which is homologous to the target locus, but comprises a sequence modification compared to the target locus. The gene targeting substrate may be produced by reverse transcription of a gene targeting message RNA (gtmRNA). The gene targeting message RNA may be folded for self-priming for reverse transcription by a reverse transcriptase. The gene targeting message RNA may in turn be the product of transcription of a gene targeting construct (GTC) encoding the gene targeting message RNA. The gene targeting construct may for example be a DNA sequence integrated into the genome of the host, or integrated into an extrachromosomal element. Following expression of the gene targeting systems of the invention, hosts may for example be selected having genomic modifications at a target locus that correspond to the sequence modification present on the gene targeting nucleotide sequence.

In some embodiments, the structure of retrons may be adapted for use in the gene targeting systems of the invention.

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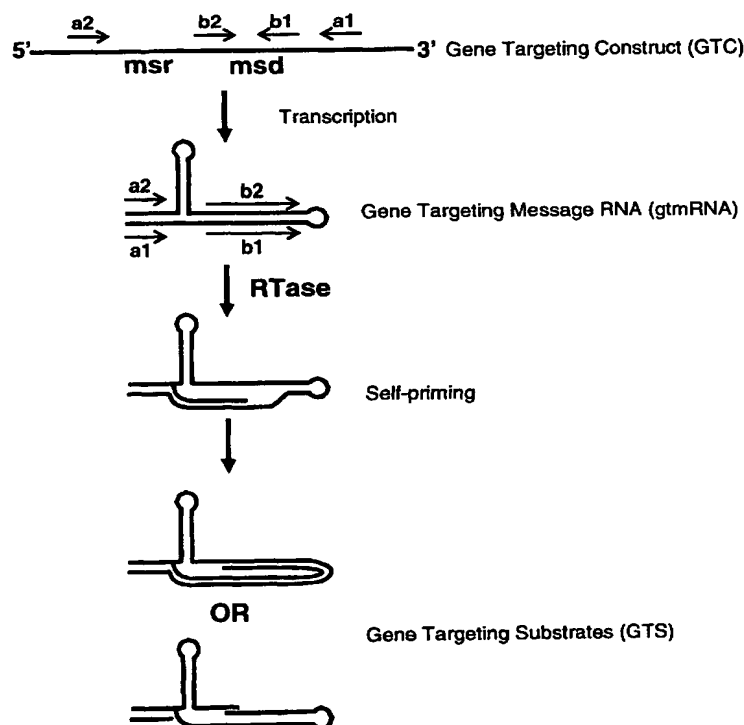
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the invention.

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SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report:  
10 June 2004

**Published:**

— with international search report

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No

P A 03/00850

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/90 C12N9/12 C12N15/11 C12N15/79 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EP0-Internal, MEDLINE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATTA H J ET AL: "Intracellular generation of single-stranded DNA for chromosomal triplex formation and induced recombination." NUCLEIC ACIDS RESEARCH. ENGLAND 15 DEC 2001, vol. 29, no. 24, 15 December 2001 (2001-12-15), pages 5140-5147, XP002253387 ISSN: 1362-4962 page 5140, right-hand column, paragraph 3; figures 1,3 page 5141, left-hand column, paragraph 1 page 5144, right-hand column, last paragraph - page 5146, right-hand column, paragraph 1	1-5, 22, 23
Y	the whole document ----- -/--	6, 8-21

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

10 September 2003

Date of mailing of the international search report

20. 01. 04

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# INTERNATIONAL SEARCH REPORT

International Application No

PO 03/00850

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J-R MAO ET AL: "Gene regulation by antisense DNA produced in vivo" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 270, no. 34, 25 August 1995 (1995-08-25), pages 19684-19687, XP002132578 ISSN: 0021-9258 page 19686, right-hand column, last paragraph - page 19687, left-hand column, paragraph 1; figure 1	6,8,9, 11-21
Y	MIROCHNITCHENKO O ET AL: "Production of single-stranded DNA in mammalian cells by means of a bacterial retron" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 269, no. 4, 28 January 1994 (1994-01-28), pages 2380-2383, XP002132577 ISSN: 0021-9258 page 2382, right-hand column, line 27 - line 31	10
A	RUSSELL D W ET AL: "Human gene targeting by viral vectors." NATURE GENETICS. UNITED STATES APR 1998, vol. 18, no. 4, April 1998 (1998-04), pages 325-330, XP001105541 ISSN: 1061-4036 page 328, right-hand column, paragraph 2 - page 329, left-hand column, last paragraph; figure 2	1-23
A	MIYATA S ET AL: "In vivo production of a stable single-stranded cDNA in Saccharomyces cerevisiae by means of a bacterial retron." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. UNITED STATES 1 JUL 1992, vol. 89, no. 13, 1 July 1992 (1992-07-01), pages 5735-5739, XP002253389 ISSN: 0027-8424 the whole document	1-23
A	EP 0 532 380 A (UNIV NEW JERSEY MED) 17 March 1993 (1993-03-17) page 9, line 20 - line 26; figure 10; example 1	1-23

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# INTERNATIONAL SEARCH REPORT

International Application No

PO A 03/00850

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INOUE M ET AL: "In vivo production of oligodeoxyribonucleotides of specific sequences: application to antisense DNA." CIBA FOUNDATION SYMPOSIUM. NETHERLANDS 1997, vol. 209, 1997, pages 224-233; discussion 233 - 234, XP001154200 ISSN: 0300-5208 -----	
A	FUJIOKA K ET AL: "Targeted recombination with single-stranded DNA vectors in mammalian cells." NUCLEIC ACIDS RESEARCH. ENGLAND 11 FEB 1993, vol. 21, no. 3, 11 February 1993 (1993-02-11), pages 407-412, XP001155003 ISSN: 0305-1048 -----	
P,A	KREN BETSY T ET AL: "The application of DNA repair vectors to gene therapy." CURRENT OPINION IN BIOTECHNOLOGY, vol. 13, no. 5, October 2002 (2002-10), pages 473-481, XP002253393 ISSN: 0958-1669 the whole document -----	1-23
P,A	TAUBES GARY: "Gene therapy. The strange case of chimeraplasty." SCIENCE. UNITED STATES 13 DEC 2002, vol. 298, no. 5601, 13 December 2002 (2002-12-13), pages 2116-2120, XP002253394 ISSN: 1095-9203 the whole document -----	1-23

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CA 03/00850

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 1-4, 18-20 are (inter alia) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
  
1-6, 8-23

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6,8-23

method of modifying a target nucleic acid within a genome of a host involving a reverse transcriptase, gene targeting constructs for said method, vectors, hosts

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2. claim: 7

reverse transcriptase having a nuclear localization signal sequence

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# INTERNATIONAL SEARCH REPORT

International Application No

PO A 03/00850

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0532380	A	17-03-1993	US 5436141 A 25-07-1995
			CA 2075515 A1 01-03-1993
			DE 69228098 D1 18-02-1999
			DE 69228098 T2 01-07-1999
			EP 0532380 A2 17-03-1993
			JP 3140206 B2 05-03-2001
			JP 6217776 A 09-08-1994
			US 5849563 A 15-12-1998
			US 2002048802 A1 25-04-2002
			US 5434070 A 18-07-1995

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Figure 1

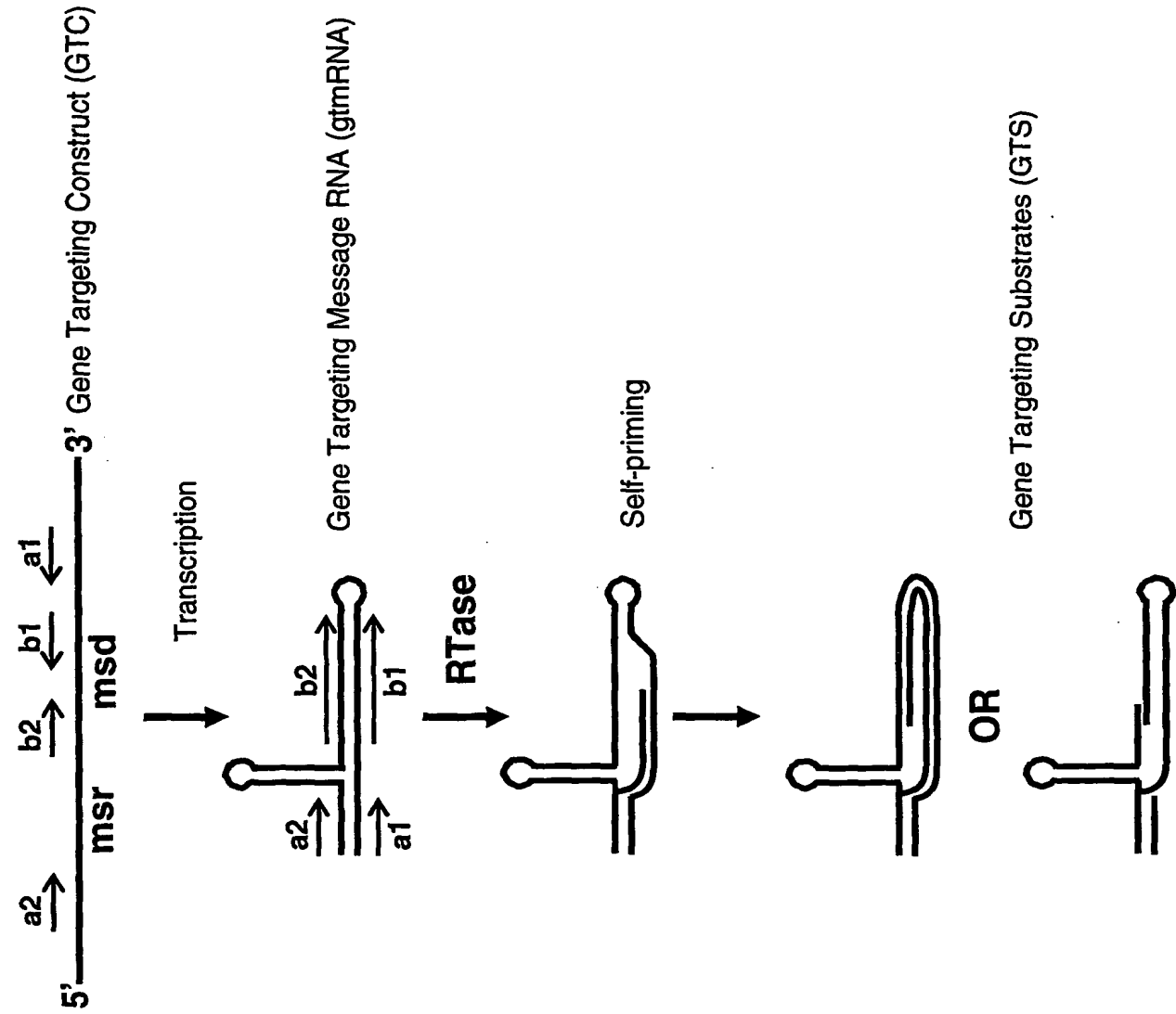


Figure 2

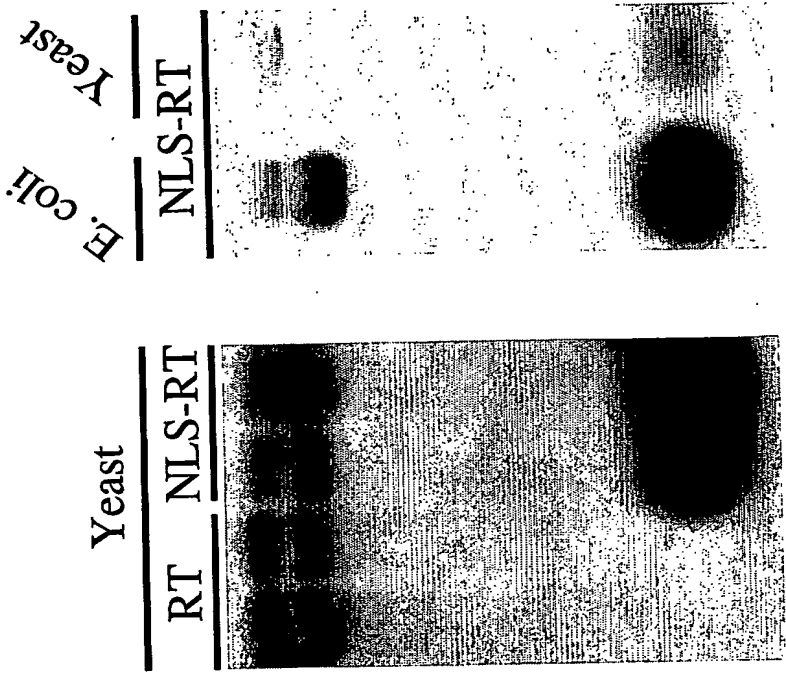
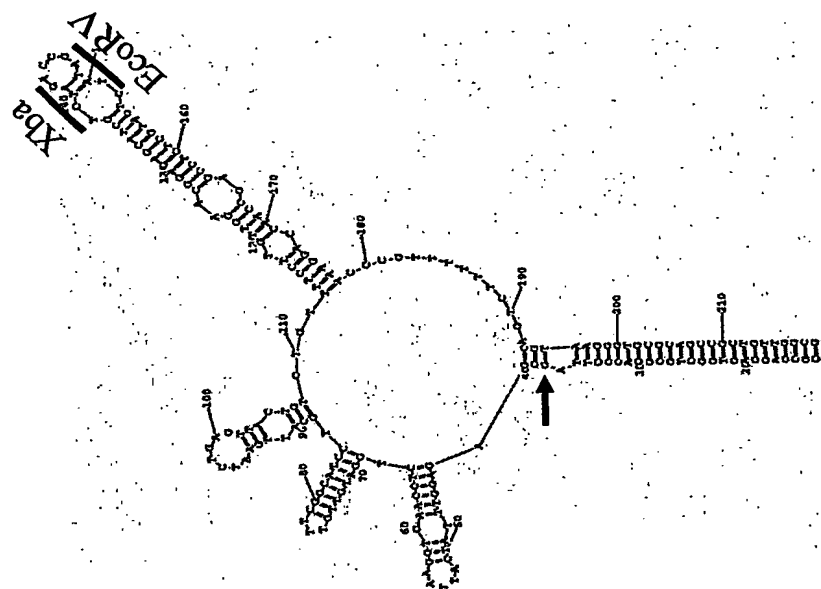


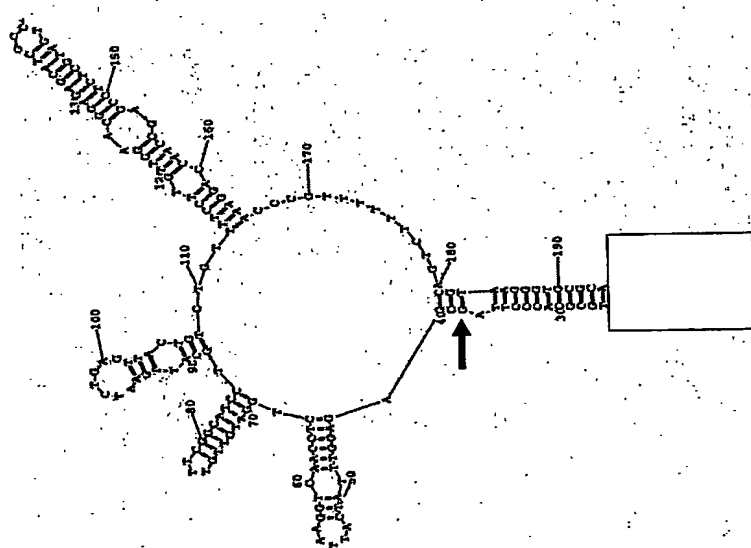
Figure 3a



**Figure 3b**



### Stem 3



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Figure 4

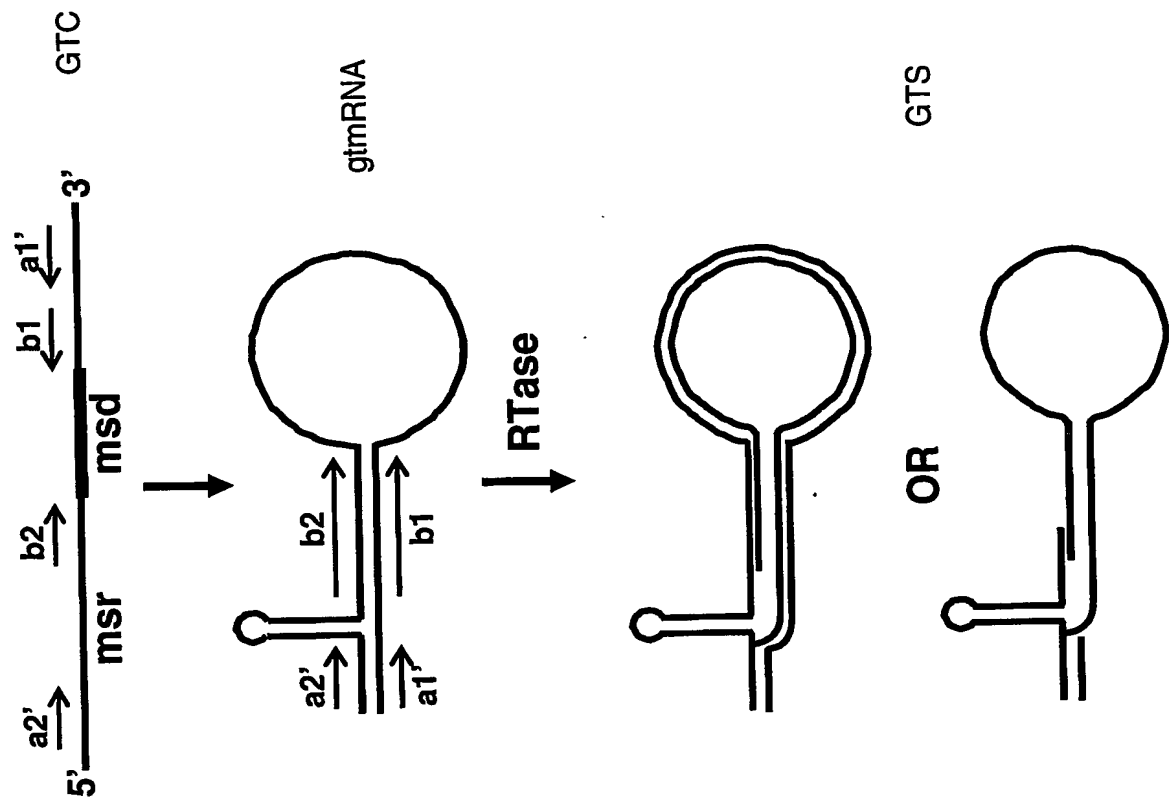


Figure 5

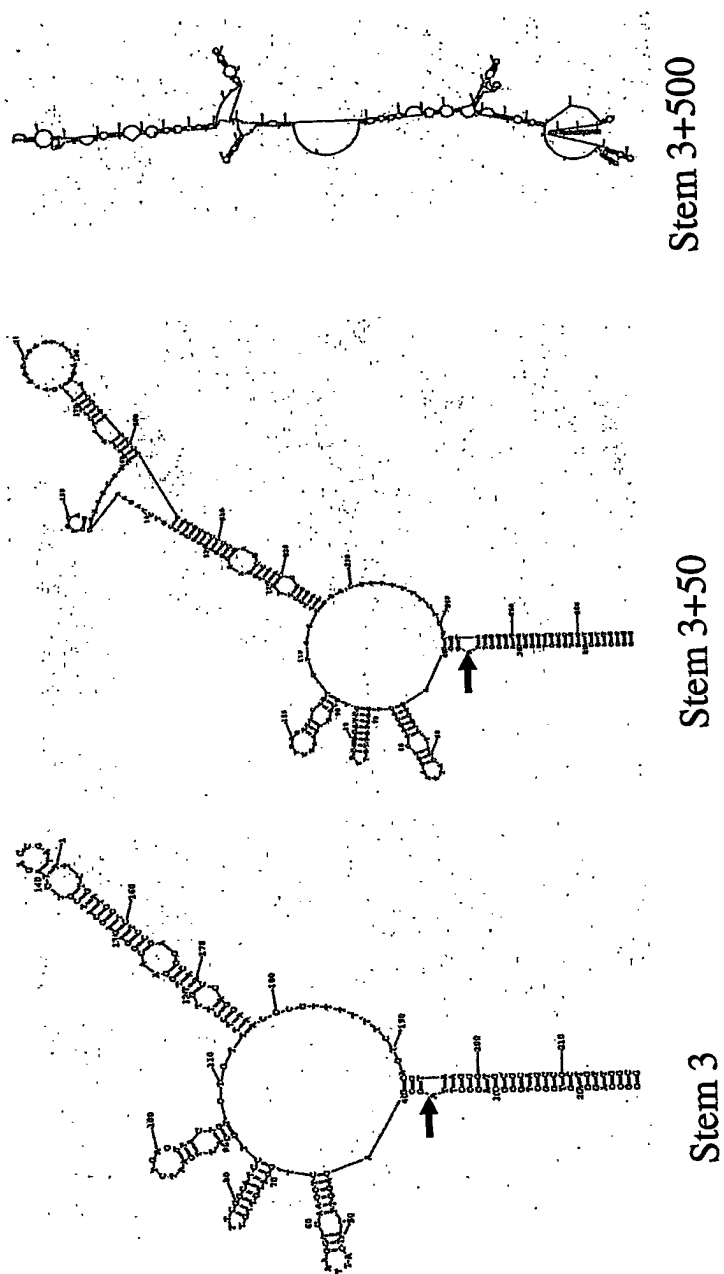


Figure 6

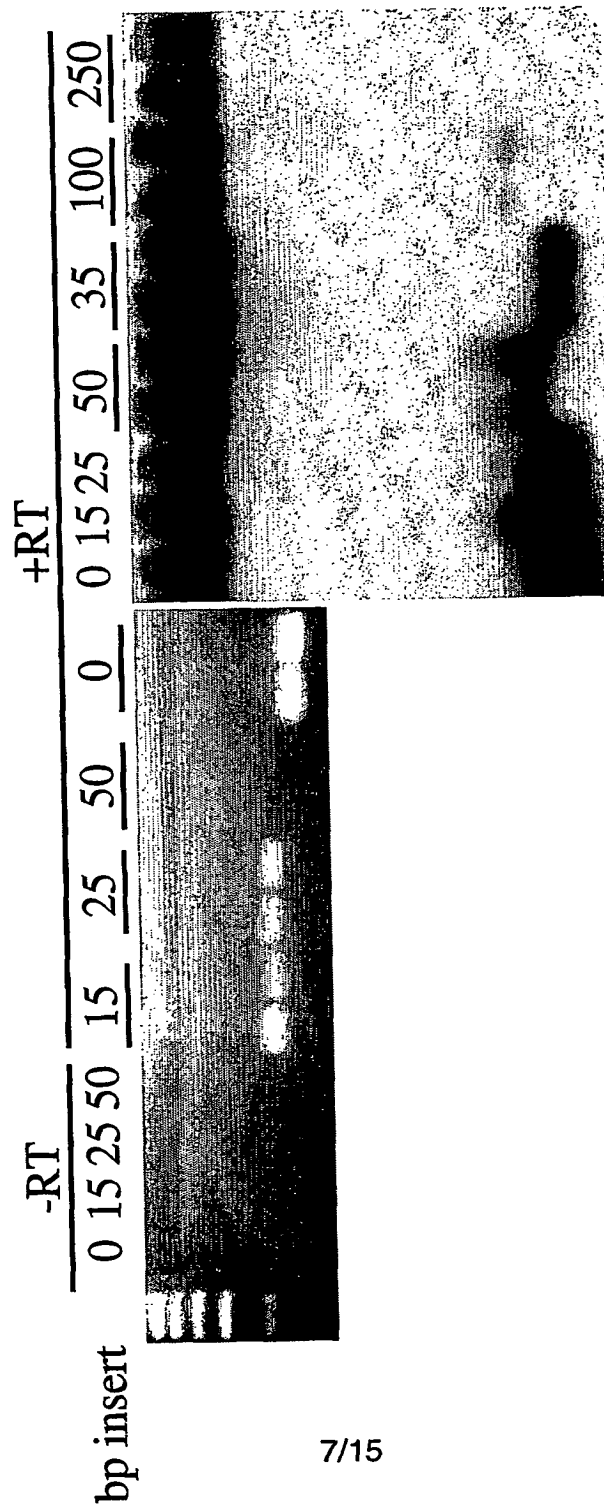




Figure 7

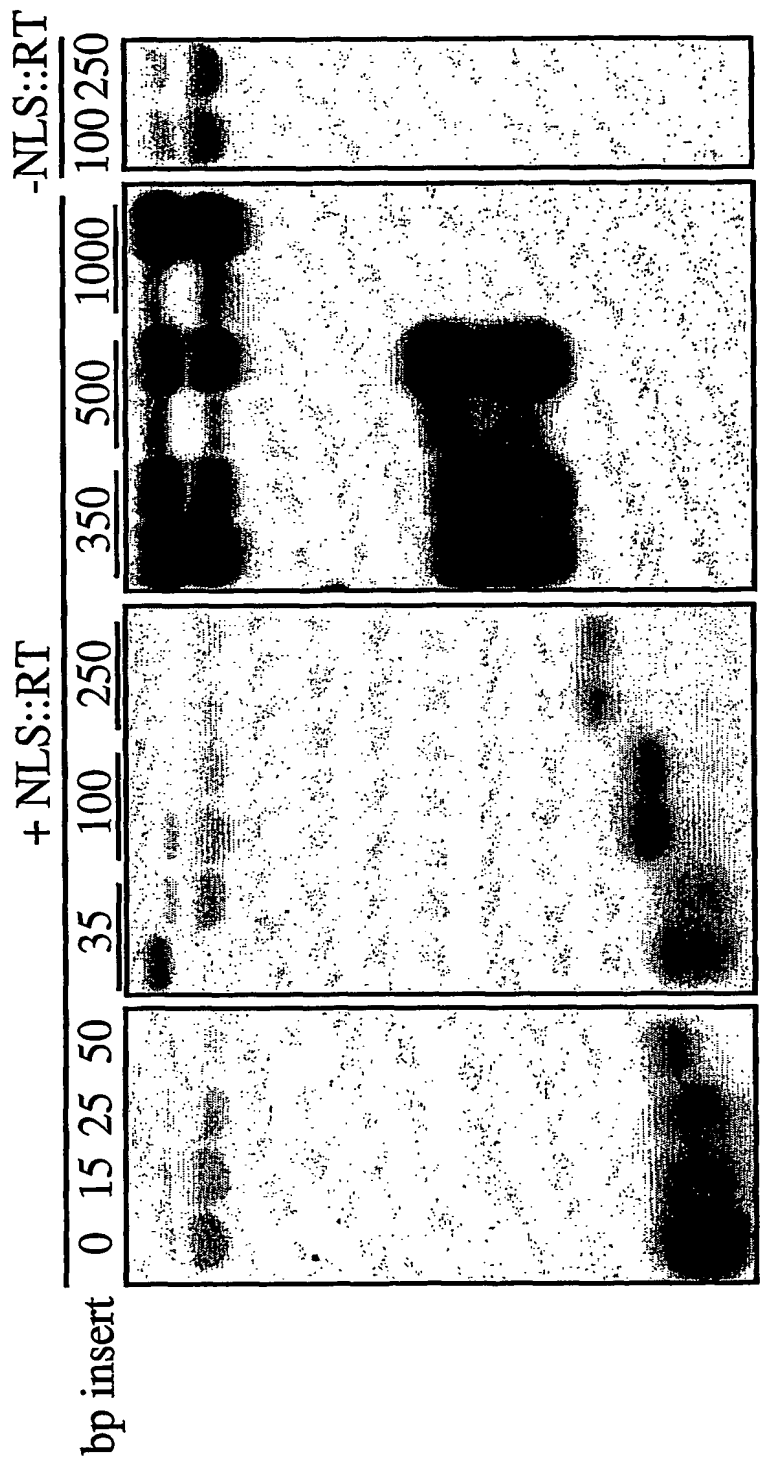
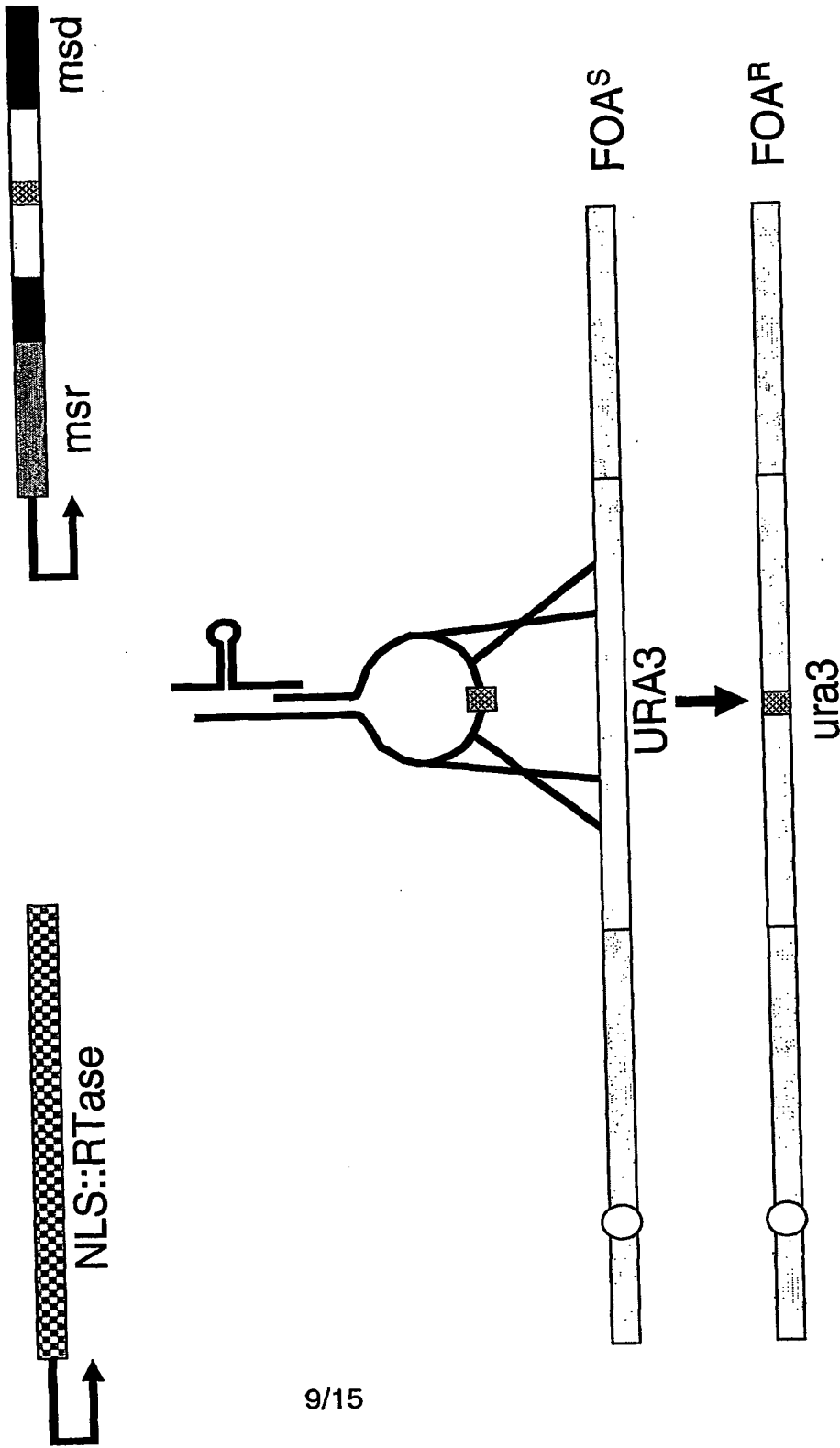


Figure 8



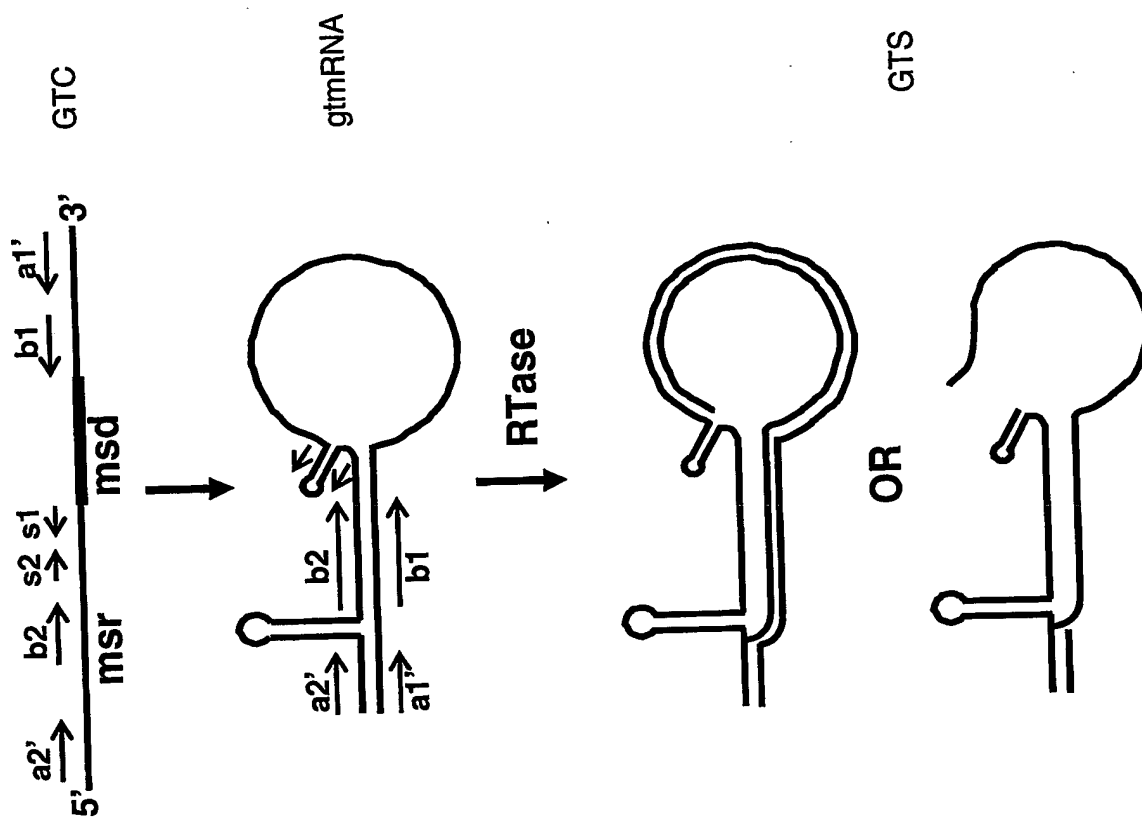


Figure 9

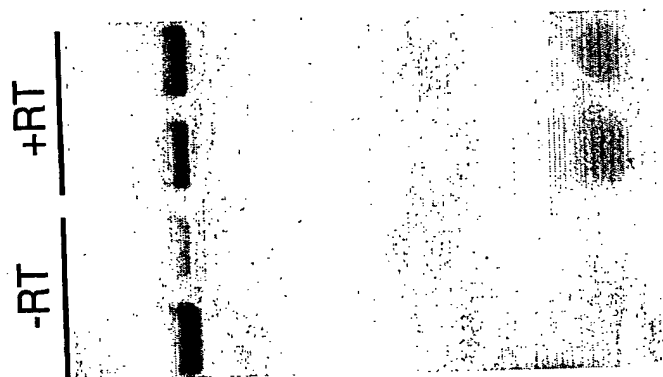
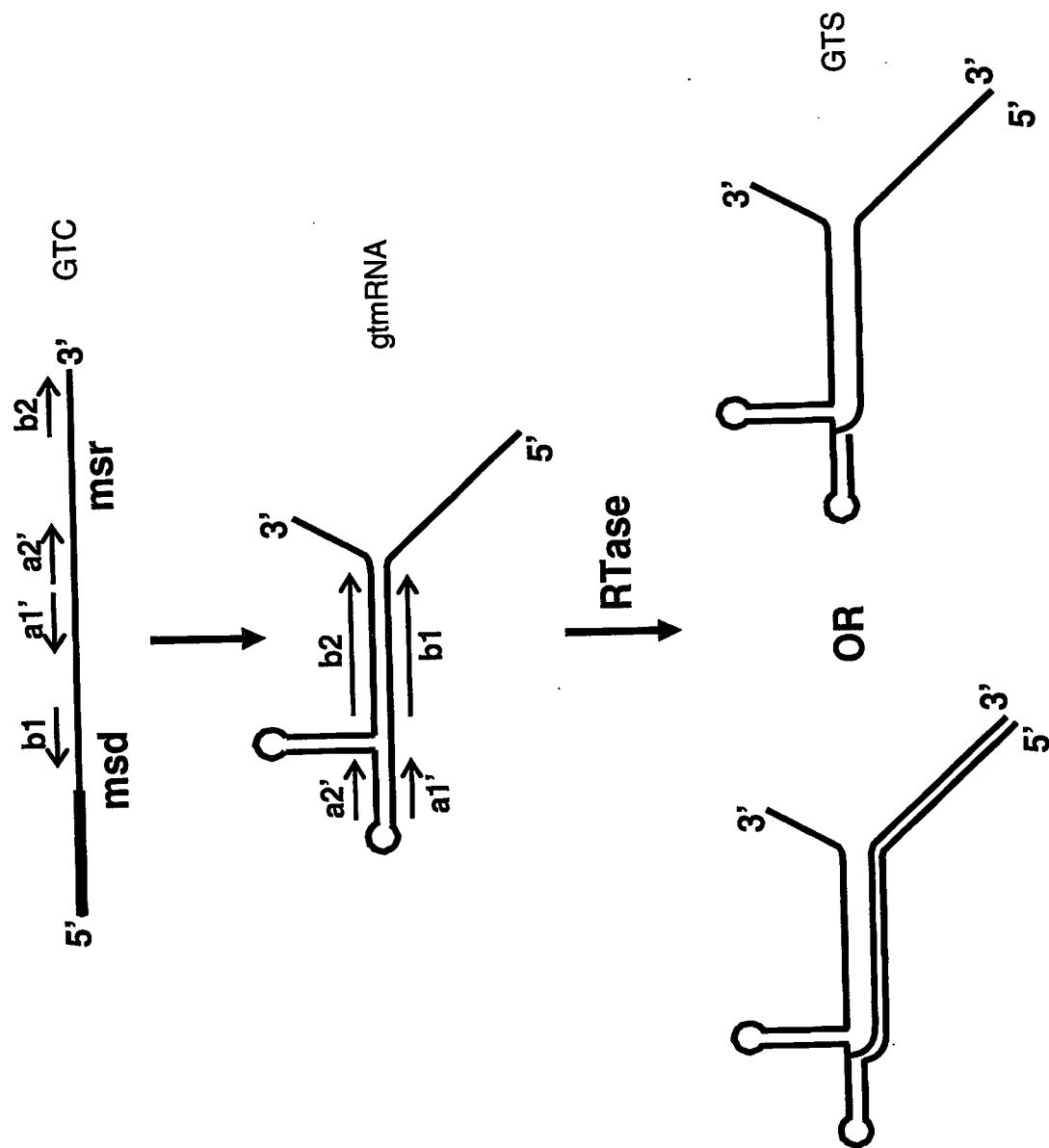


Figure 10

Figure 11



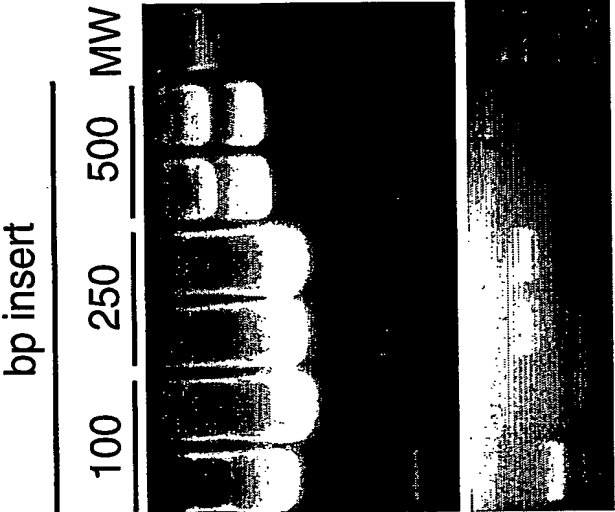


Figure 12

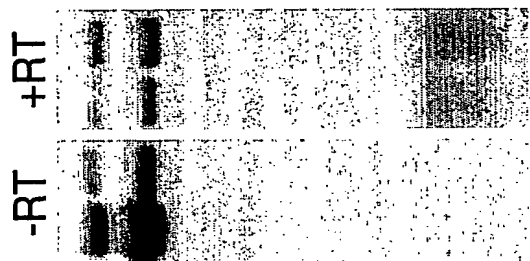


Figure 13

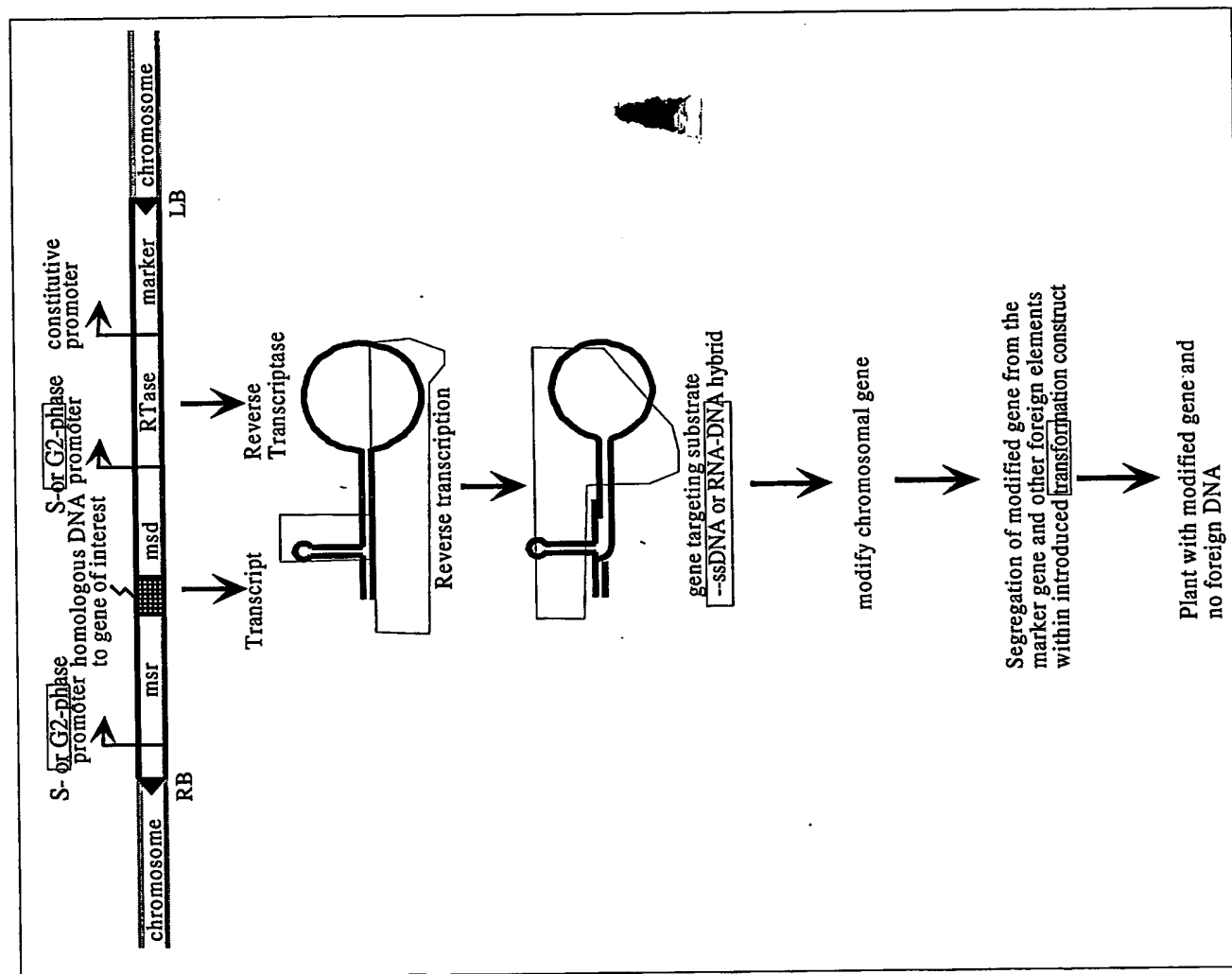


Figure 14



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